PROJECT NUMBER : 1902
PROJECT TITLE : Tobacco Microbiology
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I. TOBACCO MICROBIOLOGY

A. Objective: To develop methods and to evaluate the microflora in tobacco materials.

B. Results:

1. Evaluation of the SOP for Yeast Enumeration

The effects of the addition of varying concentrations of Oxgall to HC agar (HCA) were investigated for its ability to reduce the size and/or number of mold colonies in a mixed population of molds and yeasts. A mixed population of actively growing yeast (R. glutinis) and mold (A. versicolor) was plated on HCA with 0, 1, 1.5, 2, 2.5, 3, 3.5, and 4% Oxgall. No effects were observed from the growth on the plates with Oxgall, regardless of its concentration, when compared to the control plates (1).

2. Evaluation of the SOP for Mold Enumeration

Spore suspensions of A. niger were heated at 50°, 65°, and 80°C for 30 minutes and plated on Potato Dextrose Agar (PDA) to determine their heat tolerance. This could be useful in the separation of mold spores and mycelia. After the incubation period no growth was seen on the plates from the spores held at 65° and 80°C. Growth was obtained from the spores held at 50°C; however, the count was slightly lower compared to the unheated, control spore suspension (2).

3. Microbiological Support

Burley top coat (BUTC) samples with varying levels of ethanol were submitted by Flavor Technology personnel and analyzed for total microbial counts. No bacterial, mold or yeast growth was observed from BUTC samples prepared at the OC after seven days of storage at room temperature (3).

Studies were initiated to determine the growth characteristics of a yeast culture, Candida utilis, as requested by the Tobacco/Smoke Relationships Project personnel.

A control and three month storage sample of St. John's Bread liquid flavoring was microbially analyzed as requested by Flavor Technology personnel. The samples contained <10 and <100 bacteria/ml at time zero and after three months, respectively. No mold and/or yeast growth was detected from either sample regardless of storage time (4).

C. Plans: (1) Issue appropriate memos. (2) Continue experiments to establish a method for counting mold mycelial growth vs. mold spores in a mixed population.

D. References:

Gaines, O. Notebook No. 9093, pp. 37-38.

Chadick, D. Notebook No. 9044, p. 44.

Chadick, D. Notebook No. 9044, p. 43.

Jones, J. Notebook No. 8590, p. 165.

II. CONTROLLED TOBACCO SPOILAGE

A. Objective: To spoil tobacco under controlled conditions and determine changes in low molecular weight acids and total reducing sumgars produced by mold growth.

B. Results: An additional DBC bright tobacco experiment was conducted. Samples of DBC bright (n=2) were stored at 97% RH and 26°C and allowed to spoil. A portion of the tobacco was analyzed immediately after visible growth was observed (6 days). The remainder of the tobacco was held under the storage conditions an additional six days, 12 days total storage. No increases were seen in low molecular weight acids i.e. citric, malic, oxalic, lactic, propionic, or butyric; however, decreases of 80% and 60% were observed in formic and acetic acids, respectively, in the moldy tobacco (day 6). A 40% decrease in reducing sugars was also seen from the just visibly molded tobacco (day 6) as well as in the tobacco held for a total of 12 days. Some changes were also observed in the CO_2 evolution pattern in the moldy tobaccos compared to the control samples. Odor changes, a fermentation-type odor, were detected only 24 hours after the mold contamination was visibly noticed at day 6 (1).

C. Plans: Changes in low molecular weight acids and total reducing sugars in Oriental tobacco will be investigated.

D. Reference:

Weissbecker, L. Notebook No. 9060, p. 24.